SUPPLEMENTAL DATA

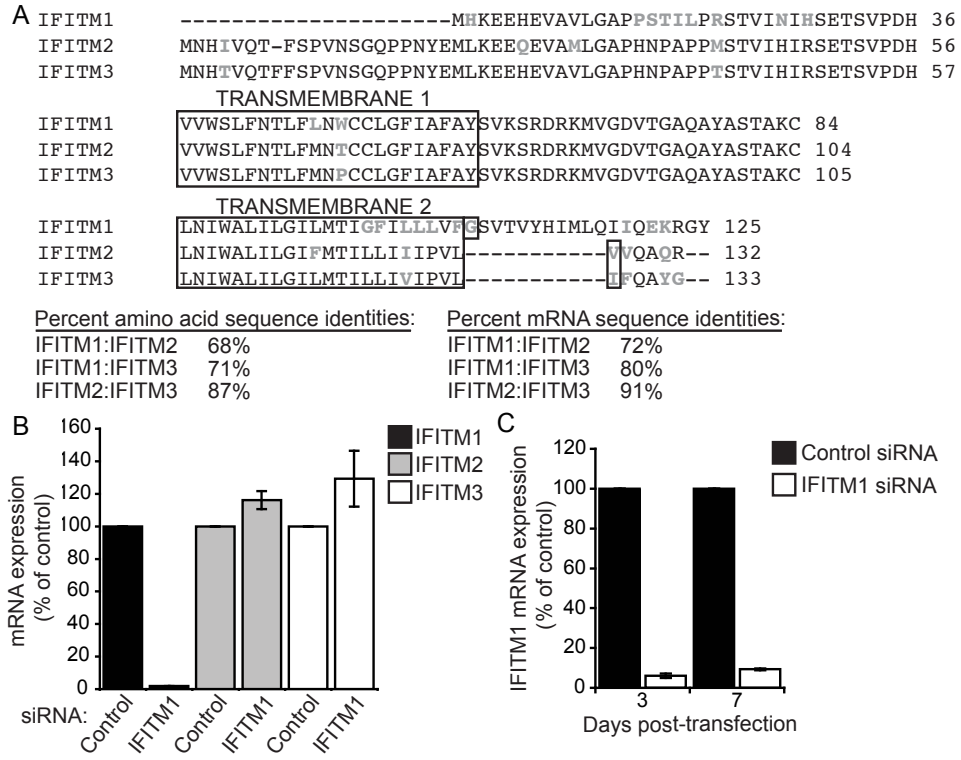

Figure SI. siRNA-mediated knockdown of IFITM1 expression in ECs is efficient and specific. (A) Amino acid sequence alignment of human IFITM1, IFITM2, and IFITM3 proteins. Amino acid residue substitutions are shown in grey. (B) ECs were transfected with control or IFITM1 siRNAs and examined by qRT-PCR 24 hours later for expression of IFITM1, IFITM2, and IFITM3. Data are represented as percent of control expression (set to 100)±SEM for each gene (*n*=3). (C) ECs were transfected with control or IFITM1 siRNAs and knockdown of IFITM1 mRNA expression was assessed by qRT-PCR at 3 and 7 days post-transfection. Data are represented as percent of control expression (set to 100)±SEM for each time point (*n*=3).

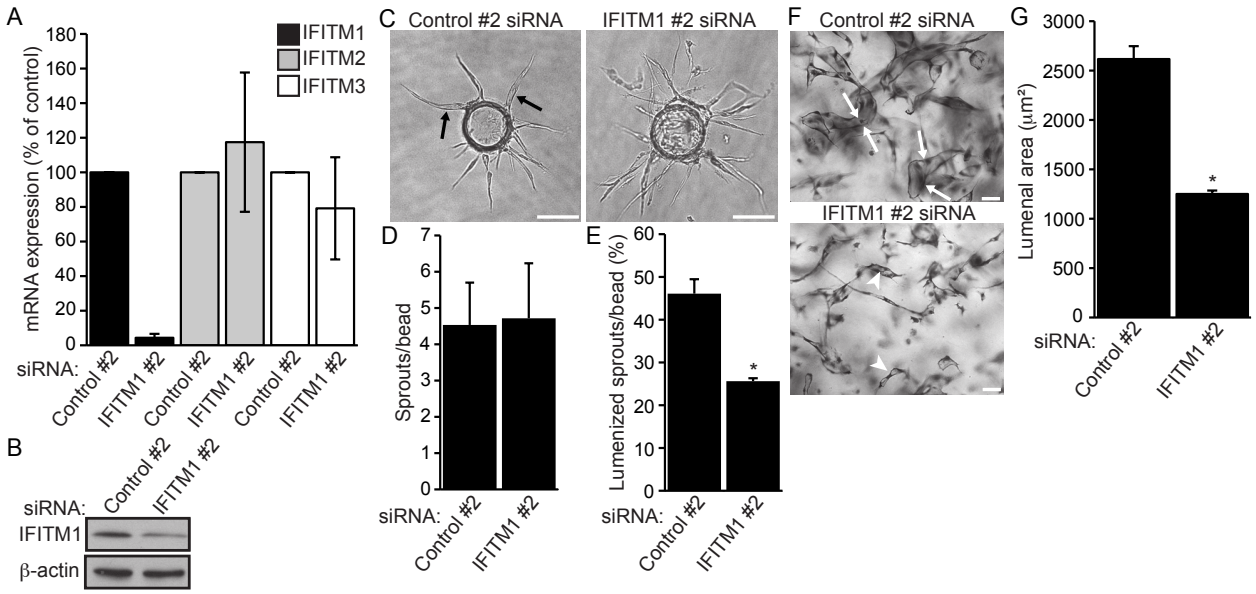

Figure SII. Knockdown of IFITM1 using an independent siRNA confirmed requirement for IFITM1 during EC lumen formation. (A) ECs were transfected with control #2 or IFITM1 #2 siRNAs and examined by qRT-PCR 48 hours later for expression of IFITM1, IFITM2, and IFITM3. The IFITM1 #2 siRNA was specific for IFITM1 and did not significantly affect the expression levels of IFITM2 or IFITM3. Data are represented as percent of control expression (set to 100)±SEM for each gene (*n*=3). (B) ECs were transfected with control #2 or IFITM1 #2 siRNAs and knockdown of IFITM1 protein was examined by western blot 72 hours later. Blots were probed for β-actin as a loading control. (C-E) ECs transfected with control #2 or IFITM1 #2 siRNAs were seeded into angiogenesis assays and analyzed for the number of sprouts (D) and percentage of lumenized sprouts (E) 6 days later. Values are means±SEM (*n*=3). **P*<0.05. Images from a representative experiment are shown (C). Arrows indicate vessels containing a continuous intercellular lumen. Scale bars, 100 μm. (F-G) ECs transfected with control #2 or IFITM1 #2 siRNAs were seeded into lumenogenesis assays and fixed 48 hours later. Representative images of toluidine-stained cultures are shown. Arrows point to large multicellular lumens in control cultures (top panel). Arrowheads indicate small intercellular lumen structures in IFITM1 knockdown cultures (bottom panel). Scale bars, 50 μm. (G) Assays were quantified and data are shown as the mean lumenal area±SEM (*n*=3). **P*<0.05.

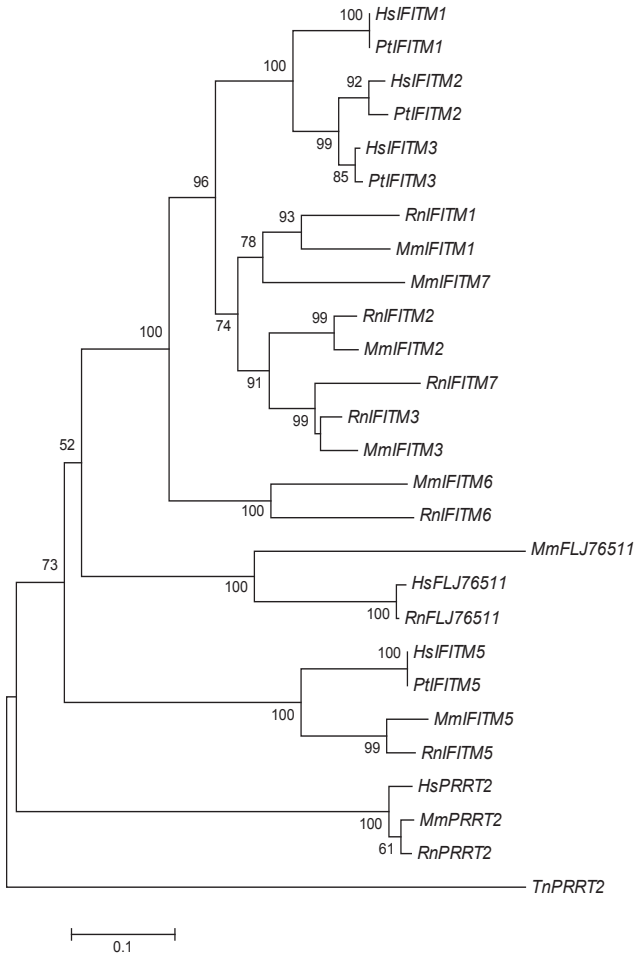

Figure SIII. Phylogenetic tree for amino acid sequences of the *IFITM* gene family. Bootstrap values ≥ 50% are shown. Scale bar shows *p*-distance of amino acid sequences. *Hs, Homo sapiens; Pt, Pan troglodytes; Mm, Mus musculus; Rn, Rattus norvegicus; Tn, Tetraodon nigroviridis* (outgroup).

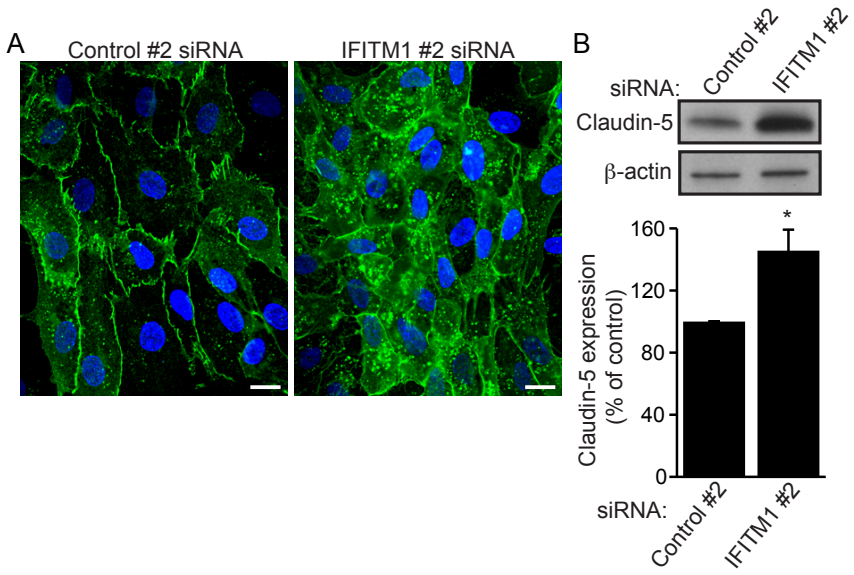

Figure SIV. Loss of IFITM1 causes aberrant cytosolic accumulation of claudin-5 protein. (A) ECs transfected with control #2 or IFITM1 #2 siRNAs were immunostained for claudin-5 (green) and nuclei (DAPI, blue). Scale bars, 10 μm. (B) ECs were transfected with control #2 or IFITM1 #2 siRNAs and claudin-5 protein expression was examined by western blot 72 hours later. Blots were probed for β-actin as a loading control. Densitometry values normalized to β-actin are expressed as percent of control±SEM (*n*=4). **P*<0.05.

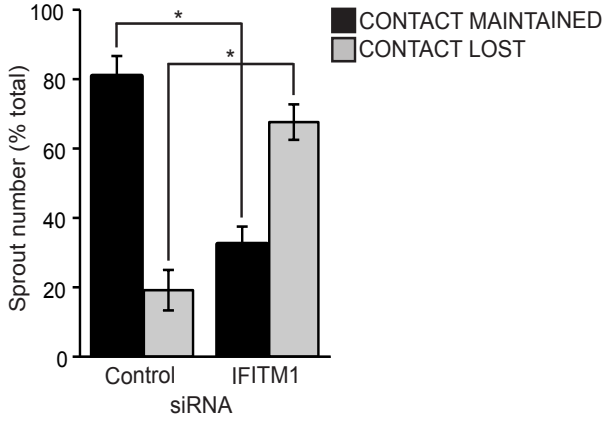

Figure SV. IFITM1 regulates junctional stability during angiogenesis in vitro. ECs transfected with the indicated siRNAs were seeded into angiogenesis assays and examined by time-lapse video microscopy on day 5. Videos were analyzed for the stability of EC-EC contacts by quantifying the number of sprouts that maintained or lost cellular contacts, expressed as a percent of the total number of sprouts quantified for each condition±SEM (n=2). *P<0.05.

Video SI. ECs transfected with control siRNA were seeded into lumenogenesis assays and examined using time-lapse video microscopy. Images were taken every 10 minutes for 69 hours, after which the collagen gels contracted and collapsed. Movies are shown at a speed of 18 frames per second.

Video SII. ECs transfected with control #2 siRNA were seeded into lumenogenesis assays and examined using time-lapse video microscopy. Images were taken every 10 minutes for 42 hours, after which the collagen gels contracted and collapsed. Movies are shown at a speed of 18 frames per second.

Video SIII. ECs transfected with IFITM1 siRNA were seeded into lumenogenesis assays and examined using time-lapse video microscopy. Images were taken every 10 minutes for 69 hours, after which the collagen gels contracted and collapsed. Movies are shown at a speed of 18 frames per second.

Video SIV. ECs transfected with IFITM1 #2 siRNA were seeded into lumenogenesis assays and examined using time-lapse video microscopy. Images were taken every 10 minutes for 42 hours, after which the collagen gels contracted and collapsed. Movies are shown at a speed of 18 frames per second.